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Assessment of Soil Quality of Serapuim Forest Irrigated with Secondary Treated Wastewater

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Abstract

Chemical, physical, and microbial analysis of soil are one of the important study with chemical, physical, and microbial analysis, to indicate soil quality, safety, and healthy for plants, field animals, and humans. The numbers of soil microorganisms differ greatly; they are primarily helpful for the healthy growth and development of field flora, animals, and humans. The goal of this study is to assess soil quality irrigated with secondary-treated municipal wastewater during a 20-year period in Serapuim Forest, which is located in Egypt's Ismailia Governorate. The assessment will be based on a set of chemical, physical, and biological indicators. In this study, 36 soil samples (30–60 cm depth) were collected from 6 sites represented by the symbols: A, B, C, D, E, and P in the Serapuim Forest, Ismailia Governorate. The total samples were analyzed using standardized methods to eliminate multi-collinearity. Principal component analysis (PCA) was used to reduce the dataset into new variables and estimate relative weights (Wi) and soil indicators (Si), which were then used to calculate the soil quality index (SQI). Principal component scores and assessments of soil characteristics were used to determine the soil quality for each area. On the other hand, several techniques to isolate and identify microbes have been described. The total microbial count and how many microorganisms are present in samples is one of the key indicators in the field of soil health. Because the count of microorganisms and their types should not exceed specific guidance values it is vital to keep track of the overall number of microbes and their types. In this study, the total count of microbes was estimated, in addition to identifying a number of pathogenic microbes. According to the results of SQI at the Serapuim Forest, the soil has a different SQI for each site, ranging from good to moderate to low. On another way, it is contaminated land, and this could adversely affect the fertility of the soil, and soil health.

Keywords: Serapuim Forest; microbial analysis; soil quality index; total count; treated wastewater

Introduction

Because of the scarcity of freshwater, the use of treated wastewater (TWW) for irrigation has attracted international interest. The impact of continuous TWW applications on soil quality, as well as adequate measurement and monitoring systems, remains unclear. Several searchers have investigated the effects of treated wastewater irrigation on soil properties throughout time [1]. Mentioned that no changes in soil biological and biochemical parameters after 3 years of irrigation with a tertiary-treated household effluent; however, [2] reminded that changes in soil biological and biochemical parameters after 3 years of irrigation with a tertiary-treated household effluent after 10 years of treated municipal wastewater irrigation. Irrigation with treated wastewater produces a variety of outcomes in terms of soil quality [3 and 4].

Irrigation of arable areas with treated city wastewater was introduced in the 1970s, when the

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first wastewater treatment plants were built on the Mediterranean island of Mallorca.

This study is one of many contributions that try to assess and evaluate the soil quality and healthy, of Serapuim Forest soil that was irrigated with treated wastewater, via collecting some samples for soil characteristics by chemical, physical, and biological analysis. The soil quality index (SQI) is defined as the soil's ability to supply plants with the nutrients they require throughout their growth phases (within the ecosystem) [5–7].

In some cases, determining soil quality necessitates a number of soil attributes. Because some of these variables are redundant, being able to identify critical parameters/variables can help to minimize the time and expense of in situ and laboratory tests, as well as improve models and processes for Spatio-temporal soil assessment [8]. Principal component analysis (PCA) is one of the most extensively used approaches for minimizing the number of variables by selecting those that are most significant in the data. The Soil Quality Index (SQI) is a measure of the regional variability of soil chemical and physical characteristics [9]. The PCA method was applied via IBM SPSS version 25 for statistical analysis to be used in estimating the SQI of each site in the Serapuim forest soil.

Evaluation measurements of the microbial diversity of soil samples from some field were obtained; water quality was measured for reuse after treatment in agricultural projects, and determines the environmental impacts of discharging treated water from Ismailia Serapuim sewage treatment facility, which pumps about 180 thousand cubic meters of treated wastewater as part of a national project to reuse. The use of water in the woody and non-fruit crops, the aromatic and oil plants; that use their oils in many developments and economic ways [10, 11, 12 and 13].

Egypt's ministry of state for environmental affairs, in partnership with Egypt's ministry of agriculture and land reclamation, established the Serapuim plantation forest in 1998 to carry out the project "national program for the safe use of treated sewage water for afforestation."

The plantation's total area expanded from 126 hectares (300 feddans) in 2005 to 252 hectares (600 feddans) in 2010 [14]. The overall area was estimated to be 241 ha (574 feddans) in October 2012 and up till now.

Wastewater treatment in Serapuim forest: TWW is provided at no cost for afforestation operations under the national program for the safe use of treated waste water for afforestation, which is regulated by the ministry of housing, utilities and urban development (MHUUD).

According to the Serapuim plantation manager, Ismailia's TWW basins can produce 90,000-130,000 m3/day of TWW. The water is piped into the plantation from the accumulation basins. The UAE is in charge of maintaining and operating these pumps. The water is treated at a preliminary stage to remove solids and other big materials before being sent to the plantation [15 and 16].

After that, it is treated again in various stabilization ponds (basins) near the plantation.

The two levels of treatment are summarized below: the primary treatment is: the primary treatment's goal is to remove organic and inorganic solids through sedimentation, as well as any remaining floating foamy debris through skimming.

During the initial treatment, approximately 25 to 50 percent of the entering biochemical oxygen demand (BOD), 50 to 70 percent of the total suspended solids (SS), and 65 percent of the oil and grease are removed. Some organic nitrogen, organic phosphorus, and heavy metals linked with solids are also eliminated during primary sedimentation, while colloidal and dissolved constituents are unaffected.

Secondary treatment: the goal of secondary treatment is to treat the primary treatment effluent again in order to remove any remaining organic and suspended particles. Aerobic biological treatment techniques are used to remove biodegradable dissolved and colloidal organic materials. Water is pushed and oxygenated in the basins by rotors, which consumes a large amount of energy.

The major goal of this study was to assess the SQI via the PCA method and then soil health using biological parameters in the Serapuim forest of Ismailia governorate, Egypt, which had been irrigated for more than 20 years with secondary-treated municipal wastewater using selected chemical, physical, and biological parameters as indicators.

Materials and Methods:

1. Location study and samples collection:

The study area is the Serapuim forest, which is located in northeastern Egypt, within the governorate of Ismailia, roughly 16 next to the Suez channel and the Serapuim village, between latitude 30° 28' 55" and 30° 29' 8" N and longitude 32° 13' 55" and 32°

14' 25" E, as shown in Fig 1.

Six sandy soil sites A, B, C, D, E and P were collected from Serapuim Forest. 1, 2, 3, 4 and 5 are samples symbols of each site. These samples are replicates from different locations for the same site. Table 1 shows the sample sites cultivation and irrigation status.

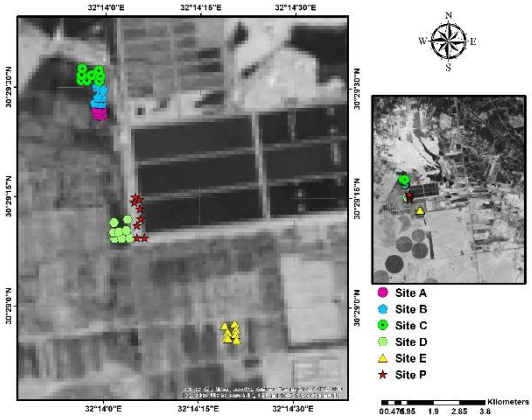

Fig.1. location of the study area and coordinates of the existing experiments in Serapuim forest, within the governorate of Ismailia.

Table (1): sample's sites, cultivation and irrigation status.

soil sample symbols	cultivation and irrigation status. (at the time of sampling)
A	cultivation since 2014 irrigation submergence
B	cultivation since 2010 dripping irrigation
C	cultivation since 2012 dripping irrigation
D	cultivation since 2002 irrigation dripping
E	cultivation since 2006 irrigation dripping
P	non cultivated

2. Chemical analysis [17, 18, 19 and 20]

The laboratory analyses were applied to characterize the physical, chemical and available nutrient condition. The collected soil samples were air dried, crushed and sieved through 2.0 mm sieve. Then physical and chemical properties were determined for the soil samples as follows:

Practical size distribution was carried out according the international pipette methods and sodium hexameter phosphate, organic matter content was determined by ferrous sulphate ammonium 0.5 N modified.

Soil reaction (pH) was determined in soil: water suspension [1:5 soil water] using pH mater with glass electrode as described. Electrical conductivity as well as soluble ion were determined in soil water extract (1: 5 soil: water) as described. Soluble calcium and magnesium were determined using versioned method while, soluble sodium and potassium were determined by using flame photometer.

The soluble anions {CO_3^- and HCO_3^-} were measured using (HCl 0.01N), (phenolphthalein and methyl orange) indictors from total soluble cations for each soil sample. Determined chloride ion by Mohr's method using ($AgNO_3$) 0.01N.

Available nitrogen was determined by micro kjeldahl distillation method model U.D.K no: 127 using boric acid at 5% and NaOH at 40% as described by Black**.**

Available phosphorus was determined using olsen's sodium bicarbonate extraction method.

Available potassium was extracted using {1M ammonium bicarbonate (NH_4HCO_3), 0.005 M diethylene triamine penta acetic acid (DTPA),pH of 7.6} extraction solution.

Available Fe, Zn, Mn, Cu, Ni and Cr were extracted using {1M ammonium bicarbonate (NH_4HCO_3), 0.005 M di-ethylene triamine penta acetic acid (DTPA),pH of 7.6} according to Page et al (1982), extraction solution were determined against a standard using a ICP instrument prodigy7. The ICP specified by optical design high energy echelle polychromator connected with a detector CMOS.

2.1 Determining soil pH

The pH of the suspension was determined using a pH meter. The determination of the pH was carried out in duplicate and the average results were recorded.

2.2 Determining soil potassium

The wavelength of the spectral line available for determining K by flame spectrometer is 766.5 nm. Detection limit and sensitivity will vary with instrument and type of flame used. Air-acetylene or air-propane is the most common flame types used. Specific techniques must be worked out for each instrument as recommended in the operation manual.

2.3 Determining soil elements and main salts:

The total N and some elements namely: Cu, Zn, Pb, Ni, Cd, Cr, Mo, P, K, Ca and Mg were measured according to **[21 and 22]**.

3. Physical analysis [23, 24 and 25]

3.1 Determination of moisture content

Two crucibles were dried in the oven for 24 hours at 105^{0C}. They were cooled in the desiccators and their weights were taken separately. 1 gram of soil

sample was weighed with each of the crucibles. The samples were dried in an oven at 105ºC for 24 hours. The crucibles were then transferred into a desiccators and the sample were allowed to cool down. The crucibles and the samples they contained were weighed. The weight of each dried sample was calculated. The samples were heated repeatedly to constraint weights. The formula below was used to calculate the percentage of moisture in each of the soil samples.

$$\frac{\text{Loss in weight of sample}}{\text{Initial weight of the sample}} \times 100$$

3.2 Determination of water holding capacity

Using aluminium tins to dry the soil in an oven at a low temperature until it is completely dry, weigh the tin by itself, and note the weight: Mc. figure out the volume of the tin, and note the volume: Vc. plug the holes and fill with water and weigh it in grams. Weigh the wet soil, and note the weight: Mt. compute the holding capacity in % VWC as calculated below.

$$\mathbf{Mw = Mt - Ms}$$

Where:

Mw is the mass of the water in grams.

Mt is the total mass of the tin and wet soil in grams.

Ms is the total mass of the tin and dry soil in grams.

Note that grams of water are the same as millilitres of water, so you can use them interchangeably.

$$\mathbf{Vw = Mw}$$

$$\mathbf{\text{holding capacity (VWC \%)} = Vw / Vt \times 100}$$

Where:

Vw is the volume of the water.

Vt is the total volume of the saturated soil.

If you've filled the tin to the top with saturated soil then this is the volume of the tin.

3.3 Determination of organic matter content

Two crucibles were dried in an oven at 105ºC for 24 hours. They were cooled in desiccators and their weights were taken separately. 1 gram of oven dried soil sample was weighed within each of the two crucibles. Each sample was heated on a bunsen burner for 30 minutes, with occasional stirring using a mounted needle. The crucibles were transferred into desiccators and the sample in it was cooled down. Each crucible was weighed together with the sample in it. The weights of the heated soil samples were determined using the formula below:

loss in weight of sample X 100%

The determination was done for the two samples and the average value was recorded as the organic matter content of the original soil sample.

4. Microbiological analysis [26 and 27]

The samples were subjected to microbiological analysis within 24 hours of collection. One gram of the soil samples was measured and dissolved in 9 ml of sterile distilled water and was tenfold serially diluted. The ten-fold serial dilution of the sample was prepared using normal saline as the diluent factor.

$$\textbf{Number of organisms} = \frac{\textbf{number of colonies}}{0.1} \times \frac{\textbf{dilution factor}}{1}$$

4.1. Total bacterial count (TBC) **and total fungi counts** (TFC).

As a method described, one ml after dilutions of each collected soil and Treated sewage wastewater samples were inoculated into nutrient agar media with dilutions (10^{-9} and 10^{-10}) for bacteria and potato dextrose agar (PDA) media with dilutions (10^{-6} and 10^{-7}) for fungi. The pour plate method was used for inoculation. The inoculated bacterial plates were incubated at 37°C for 48 hrs and fungi plates were incubated at 28°C for 72 hrs.

4.2. Total coliform counts (TCF) **and total faecal coliform counts** (TFCC).

In soil samples; the coliform counts were determined by the most probable number (MPN) technique (APHA, 1995) while, total and faecal coliform groups were determined by multi-tube fermentation methods (UNICEF, 2002). Presumptive test analysis was done using macconkey broth to enumerate total coliforms while confirmatory test analysis of the samples was done using brilliant green broth to enumerate faecal coliforms. Mostly conditions were used in this test are 28ºC, 10^{10} tenfold for serial dilution.

4.3. Isolation of actinomycetes

One ml after dilutions (10^{-3} and 10^{-4}) of each collected soil and wastewater samples were inoculated into casein starch agar (CSA) medium for actinomycetes. The pour plate method was used for inoculation. The inoculated bacterial plates were incubated at 30°C for 72 hrs.

4.4. Enumeration of spore-forming bacteria

The procedure of (ISO, 2002) 1 ml of water was taken into a sterile blender jar and blend with 90 ml sterilized peptone (0.1%) pipet 10 ml to each of two large 22mm diameter tubes making sure sediment is

transferred also. Place the thermometer in one of the tubes and cap the other loosely. Heat the tubes in the 82-83°C water bathe with agitation, when the tube with thermometer reaches 80°C; hold both tubes for 20 minutes at that temperature. Serial dilutions from 10^{-8} to 1^{-9} were plated on plate count agar (PCA) and incubated at 30°C for 3 days.

4.5. Total count of N_2 fixers [28]

Jensen media is used for isolation of *Rhizobium* spp. and *Azotobacter* spp. One ml after dilutions (10^{-5} and 10^{-6}) of each collected soil and treated sewage wastewater samples. The inoculated plates were incubated at 37°C for 72 hrs.

4.6. Enumeration of *Escherichia coli* bacteria

A 0.1ml aliquot of four-fold serial dilution of the sample was inoculated onto macconkey and nutrient plates, with pour plate method. The plates were incubated at 37°C for 24-48 hours. Observations were made for development of colonies. The visible colonies on the plates were counted and recorded.

4.7. *Clostridium* spp. count

For ***Clostridium*** spp. count, serial dilutions of soil samples were made from 10^{-1} to 10^{-3}. Decimal volumes of 1ml and 0.1ml of each dilution were aseptically transferred to 10 ml of sterile plates containing medium and incubated at 35°C.

Plates were examined for single colonies between 24 to 48 hours.

4.8. *Pseudomonas* count

Pseudomonas spp. was estimated using appropriate dilutions of analysed samples (10^{-1} and 10^{-3}) for agricultural wastewater filtered in duplicate through 0.45-µm cellulose-acetate filters (millipore, USA) and then placed on a pseudomonas isolation agar (PIA, BD diagnostic systems, USA) and incubated at 37°C. Typical pseudomonas colonies appearing blue-green on PIA agar plates were enumerated as total Pseudomonas counts (TPC).

4.9. *Salmonella* spp. count

The count was performed by inoculating plates containing salmonella shigella agar media (SSA) with 1mL of the samples and incubated for 18 h at 37°C., plates showing bacterial growths from each site were streaked onto selective agar plates. The plates were incubated at 37°C for 72h.

Morphological and biochemical features of bacteria remain important in the identification and classification of those organisms. Isolates are classified by morphological features on the basis of many characteristics; Cell shape, nature of multicellular aggregates, motility, formation of spores, and reaction to the gram stain are important, also including the shape and colour of bacterial colonies, are not always constant and can be influenced by environmental conditions. Important in the identification of a genus and species of bacteria are biochemical tests, including the determination of the kinds of nutrients a cell can use, the products of its metabolism, the response to specific chemicals, and the presence of particular characteristic enzymes.

Other criteria used for the identification of some types of bacteria might be their antigenic composition, habitat, disease production, and requirement for specific nutrients. Therefore, in this study; the morphological and biochemical identification was done using experiments, according to Bergey's manual of systematic bacteriology [29].

5. Soil Quality Assessment

5.1. Analytical Methods

Statistical method conducted via method of principal component analysis (PCA) which was used to choose the most adequate indicators that would generate the data referred to as principal components (PCs). The soil quality estimate was performed by using a soil quality index (SQI) that was scored on chosen variables of the PCA and the minimum data set (MDS). The PCs used as MDS had an eigenvalue that is more than 1; the weightiest factor was selected as a PC. To calculate the soil quality index (SQI) at each sample point, the value of the chosen indicator on each PC was multiplied by its scores. The score obtained from transforming each value of indicator to standardized value using equation according to [30], because the soil indicators have different scales and units.

The soil quality was calculated by multiplying the variable's score by the weighted index [31]. According to (SQI, 2001. guidelines for soil quality assessment in conservation planning) [32], the soil quality index (SQI) formula can be used to determine the assessment of soil quality.

$$\boldsymbol{SQI} = \sum_{\boldsymbol{i=1}}^{\boldsymbol{N}} \boldsymbol{Wi} \times \boldsymbol{Si}$$

Annotation:
SQI = soil quality index
Wi = weighting factor
Si = score index

If the index value has been obtained, the soil quality (SQ) can be determined according to [33], reported that the soil quality could be classified into the following conditions: very good (0.8–1), good (0.6–0.79), fair (0.35–0.59), bad (0.20–0.34), and very bad (0–0.19), as shown in Table 2.

5.1.2. Statistical Analysis

Descriptive statistics to the soil properties of Serapuim forest include the arithmetical mean, standard deviation (Std), standardization by excel software office 2013.

5.1.3. Principal component analysis method

Then conducted PCA via IBM SPSS program version 25 by clicking the tool analyse button then clicking the dimension reduction button on the table of variables analysis. PCA procedure was used to reduce the dataset into new variables, which named principal components (PCs), also to avoid multicollinearity between the original variables. These PCs explain most of the variation existing in the original variables. Soil quality index calculation is based on PCA using equation (1) according to [33] and (Cude, C.G. 2001) [34].

$$\boldsymbol{SQI} = \sum_{i=1}^{N} \boldsymbol{Wi} \times \boldsymbol{Si} \qquad \text{Equation (1)}$$

Wi denotes the relative weight of each indication, which ranges from 0 to 1, and Si is the value of each soil indicator. The component score coefficient (CSC) derived from the principal component analysis (PCA) findings is represented by Wi. The Si readings are standardized using equation (2) according [30] because the soil indicators have different scales and units.

$$\boldsymbol{z} = \frac{\boldsymbol{x}-\boldsymbol{x}^{-}}{\boldsymbol{\sigma}} \qquad \text{Equation (2)}$$

The standardized value, the value of a soil indicator, the mean of a soil indicator, and the standard deviation (std) of a soil indicator are all represented by the letters z, x, x-, respectively. According to [35], the soil quality index (SQI) equation based on principal components (PCs) becomes the following equation (3)

$$\boldsymbol{SQI\_PC} = \sum_{i=1}^{N} \boldsymbol{CSC} \times \boldsymbol{z} \qquad \text{Equation (3)}$$

When selecting (PCs), which are represented as a weight index (Wi), choose the weighted index that has the highest value in each selected PC column from the results of the PCA method, according to [33].

Then , the comprehensive soil quality index (CSQI) is computed using equation (4):

$$\boldsymbol{CSQI} = \sum_{i=1}^{N} \boldsymbol{Variability\ of\ each\ PC} \times \boldsymbol{SQI\_PC}$$

Equation (4)

Because CSQI, calculated using z scores, is transformed into a standard normal distribution (which has a mean of zero and a standard deviation of one) using equation (5) according to [30]:

$$f(x) = \frac{1}{\sqrt{2\pi}} e - \frac{(z)^2}{2} \qquad \text{Equation (5)}$$

The letters e and z refer to the natural logarithm, equal to approximately 2.718, and the CSQI, which is calculated using z scores. Then, from the result of SQI can determine the SQ as shown in table 2.

Table 2. Classes of soil quality updated from [33].

Class No.	Numerical Values	SQ
1	0.80-1	better
2	0.60-79	good
3	0.35-0.59	moderate
4	0.20-0.34	low
5	0.0-0.19	very low

Results and Discussion

The first objective of this work consisted of evaluating the soil quality,and safety. Scientists are curious about the connection among soil quality and microbial count and function..

To carry out this study, soil properties analysis was used, which corresponds to factors of considerable influence within the selection process of the indicators that make up the soil quality index. 36 sand soil samples were collected from 6 sites; (A, B, C, D, E, and P) from Serapuim forest soil – Ismailia governorate. The analyses of soil texture, chemical, physical and microbial properties were determined in these samples to assess soil quality (SQ) using a soil quality index (SQI) via a statistical approach. SPSS version 25 software was used to determine the many routine parameters of statistical interest. Moreover, through the results of microbiological analysis, it can decide on the suitability of the soil for safe use. Texture in soil indicates the relative content of particles of varying sizes, such as sand, silt, and clay. Texture influences the ease with which soil can be worked, the amount of water and air it can hold, and the velocity with which water can enter and travel

through soil. As shown in table (2), the dominant soil in the experiment is order. The surface (0–20 cm) texture is sandy, none saline, none calcareous and pH of 6.8-7.2, with a soil organic matter (SOM) of 0.13%. Details of field capacity (FC), electric conductivity (EC); even though low but they are at adequate range and other soil properties are given in table 3.

These soil properties were linked to the soil moisture content as well as the sand content of the soil, so probably that a lower organic matter and clay content where the root's absorptive capacity is less than the plant's demand.

Table (3) Soil texture analysis of Serapuim site

Texture			EC ds/m	F.C %	Available water %
Sand	**Silt**	**Clay**			
92.50	3.28%	4.22%	1.37	5.6	4.5

Chemical Analysis

The results in tables 4 and 5 showed the laboratory chemical analyses which were carried out on soil and water samples according to approved methods, which included; main macro and micro elements, and dissolved anions and cations.

According to world health organization (WHO) guidelines; irrigation by treated wastewater had negative side on soil fertility is affected by its content of extractable micronutrients such as iron, copper, zinc and manganese.

Table (4) presents a recording of the varying values of these elements in different regions under this study. We find a decrease in Nickel in all samples, followed by copper, followed by iron, while potassium, nitrogen and phosphate elements, in order, are widely available in all soil samples, and in the middle come the elements magnesium, manganese, zinc, chromium respectively, which indicates that the soil contains the main elements needed by the plant, while at the same time it is not devoid of a percentage of toxicity, which poses a threat to human and animal health.

For example the average of NPK elements content of the soil differed significantly due to different locations. In zone A, we find that all elements are mostly increased, and then decreased significantly in other locations.

Using non completing treated wastewater leads by mg/kg nitrogen (N) recorded 24.1, 22.8, 22.9, 21.7, 22.8, and 21.1 at sites A, B, D, C, E and P respectively, although phosphate (P) noted 17.8, 18.1, 18.5, 18.8, 18.7 and 18.5 at the previous respectively sites, while potassium (K) get the highest amount results, which were 106.0, 100.7, 104.9, 101.6, 104.2 and 101.1 at the same regions.

In the same time we show the toxicity minerals recorded dangerous levels in soil samples, the average of Nickel (Ni) is 0.107 and of chrome (Cr) is 0.192.

These results confirm the observation that; the irrigation to unsafe and unhealthy soils; on the other hand, those results were in contrast with what was found by [36], who reported results close to our study.

The composition of different exchangeable and chargeable main cations and anions salts inside of the soil ecosystems also contributes to the differences in the soil properties. Table 3 presented the significant among cation and anion salts which found on studying soil samples.

Site sample A1/60 had the highest value of Cation Ca^{++} (5.36 meq/l), while site P3/60 was had the highest value of cation Mg^{++} (1.89 meq/l), in the same way we found site C3/60 had (0.76 meq/l) from cation K^{+} and equal range (0.26) in sites B1/30, B2/60, C1/60.

About anions salts, it was found that (Cl^{-}, SO_4^{--} and HCO_3^{-}) recorded the highest reads (16.2, 4.14 and 1.44 meq/l) in sites (C3/60, A1/60 and C2/30) respectively of all. From the other side, no anion CO_3^{--} is detected in any of the samples.

Results refer to soils samples were close to acidic in interactions; there were attributably higher concentrations of exchangeable anions.

Microbial analysis:

Results in tables (6 and 7) are presented determination of total count of soil microbes. The isolated microbes were grown in different mediums.

As shown in table (6); microbial diversity in wastewater was determined after sewage treatment by governorate unit in Serapuim – Ismailia, (Ismailia wastewater treatment plant laboratory daily/monthly summary report of analysis) to clarify the assessment of microbial diversity analysis in Serapuim soil. Which shows the big difference in the microbial content before (influent) and after (effluent) treatment during 2018, where the average microbe count decreased by 0.24%, indicating the high quality of the wastewater treatment process at the Serapuim facility in Ismailia.

While from the same table we find the negative difference caused by misuse and perhaps the lack of

efficiency of treatment, as we find that in the year 2020 the instability of the average microbial load after treatment throughout the months of the year, but it always increases from the year 2018 by a rate ranging between 2% to 20% and this indicated the deterioration of the health and safety of the soil has a harm impact on plants and farm animals and, of course, human health.

The average of microbial load in soil samples ranged from year 2018 to year 2020 is 15,816,856 to 49,681 respectively in the influent, the average of micro loud ranged in the effluent from 38,036 259,676, although the density and diversity of species was reduced after treatments as showed in table (5).

Table 7 is clearly to us there are safe and standard criteria of bacterial and pathogenic microbial content to assess the viability of treated sewage or wastewater for agricultural usage *E.coli* is predominant one in most countries other than faecal coliforms, total coliforms, and pathogens such as ***Salmonella*** spp.

Analytical parameters included in the wastewater reuse requirements that were examined. The use of treated wastewater in agriculture is an annex to the Egyptian code. And also from the additional burden and maximum pathogen, it is reported that total coliform 10^8-10^{10}/gm non indication that the water is polluting by stool, faecal coliforms was used as a faecal indicator in bacteriological examination for water drinking, when faecal coliforms and *E.coli* are using as indicators for bacterial and fungal pathogens.

Table 8 shows the assessments of total bacteria count (TBC), total fungi count (TFC), total coli form (TCF), actinomycetes, spore forming, and N_2 fixers in soil samples and treated sewage wastewater (TSW).

The treated sewage wastewater showed the most content of all assessments except actinomycetes when compared with any soil samples. On the other hand, the comparison among soil samples showed a discrepancy where site B recorded more content than others (3.19 cfu x 10^5 gm/soil), while site P recorded lower content than others (2.31 cfu x 10^5 gm/soil), with this results we found that the total count of coliform bacteria exceeded the permissible limits according to Maximum additional disease burden from (WHO) [3].

All samples are very close in terms of the value of the Spore forming, as we find the same average value in sites B and E (2.77 cfu x 10^5 gm/soil), which is considered the highest value, while at the same time we find that location P achieves an average of (2.04 cfu x 10^5 gm/soil), which is considered the lowest value of assessments.

Table 9 shows, on the other hand, that particular microbial species were detected in the treated wastewater, as well as a rise in the averages of hazardous and pathogenic bacteria like *Salmonella*, *Clostridium* and *Pseudomonas*, which causes their transmission and accumulation in the soil, negatively affects the suitability of the soil for cultivation when irrigated with treated wastewater, despite the fact that the number of nitrogen fixers has increased in a way that reflects the soil's fertility, which helping to cause availability of organic matter and nutrients needed by the plant.

Statistical analysis for assessment soil quality index (SQI) by PCA method

SQI in each site was determined by the multiplication of the value of the selected soil properties score (Si), which result from equation (2) with the weighted index (Wi) which result from PCA method. The weighted index is the highest value in each selected PC column. Based on the results of major component analysis (PCA), the soil properties used in the determination of soil quality index for each site were bulk density, pH, EC , and soil texture ,N, P, K, Mg, Mn ,Ni, Cr, proportion of organic matter (% OM), and microbial diversity especially pathogenic bacteria such as *Pseudomonas*, *Salmonella*, and *Escherichia coli*. Most soil quality assessment studies focus on soil physical and chemical indicators and are rarely described by biological indicators [37 and 38].

Bulk density (Bd) is an important principal component (PC) which determines root development in the plant root zone. The pH and soil texture determines the availability of nutrients for trees and plants. The soil pH and clay fraction strongly determine its cation exchange capacity (CEC) has effective in providing nutrients to plants. Pathogenic bacteria such as Pseudomonas, Salmonella negatively affects the suitability of the soil for cultivation when irrigated with treated wastewater. Thus, it affects the soil health.

Refer to equation. (1) Obtained the results of the calculations of the soil quality index using the high values of PCs in each column shown in table (10), and then obtained the results of soil quality index shown in table (11). Soil quality in site A ranged from moderate to good, site B ranged moderate to good, site C good, site moderate, site E ranged moderate to good, site P is non-cultivated soil, it is

the moderate quality. There were also variations in the soil quality index between different sampling points. This was due to the irrigation with treated wastewater, the date of cultivation, and the different types of trees in each sample soil point.

Conclusion

From previous results; the usage of treated wastewater has a direct severe influence on the physical, chemical, and microbiological characteristics of the examined soil, and soil quality, since all soil samples from Ismailia were found to exhibit a high microbial load, so that the results exceed the allowable limits according to the international organizations for food, agriculture and health. The quality of the water utilised in the irrigation procedure exceeded the world health organization's acceptable guidelines. According to this study, treatment processes are unable to eradicate harmful bacteria from wastewater and soil irrigated with water.

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Table (4): Minerals determinations of soil samples

Sampling	N	P	Mg	K	Mn	Zn	Fe	Cu	Ni	Cr
site symbol	mg/kg								mg/kg	
A1/30	24.97	17.232	1.82069	109.755	1.05255	0.8352	0.60968	0.3404	0.0312	0.1944
A1/60	22.69	17.204	1.8236	110.7	0.7713	2.304	0.5857	0.3397	0.134	0.1912
A2/30	22.07	17.172	1.82021	98.685	0.7245	2.7072	0.5812	0.3414	0.0416	0.1872
A2/60	24.43	19.008	1.82409	108.27	0.94005	6.2928	0.62418	0.3401	0.0172	0.1852
A3/30	25.12	17.456	1.8243	99.422	0.8214	2.8561	0.5911	0.3502	0.0243	0.1752
A3/60	25.34	18.752	1.83212	109.321	0.8433	2.5631	0.7124	0.3621	0.0324	0.1852
B1/30	21	17.436	1.82215	102.33	0.9306	1.512	0.52573	0.338	0.1228	0.1932
B1/60	25	18.208	1.82263	103.5	0.8181	2.0592	0.61118	0.3386	0.154	0.1908
B2/30	21.46	18.172	1.82506	100.575	0.99765	2.9808	0.60369	0.3378	0.1104	0.1968
B2/60	24.79	18.244	1.8236	96.975	1.1025	0.5184	0.65816	0.3404	0.1224	0.2
B3/30	22.99	18.128	1.82312	98.01	0.74925	0.9936	0.62867	0.3392	0.0992	0.1864
B3/60	22.13	18.956	1.82263	103.275	0.76365	2.3616	0.5822	0.3399	0.192	0.1868
D1/30	24.9	18.304	1.82603	97.56	0.44955	1.0224	0.58719	0.34	0.1016	0.1836
D1/60	24.25	18.152	1.82312	105.705	0.5949	0.1008	0.65516	0.3419	0.268	0.1912
D2/30	22.14	18.992	1.82457	103.815	0.7011	0.4176	0.64317	0.3402	0.0148	0.198
D2/60	21.38	18.672	1.82409	109.845	0.5715	2.0448	0.62118	0.3398	0.1404	0.1912
D3/30	22.12	18.341	1.82301	104.882	0.6541	2.0523	0.62116	0.3337	0.1403	0.1856
D3/60	23.15	18.772	1.84201	107.654	0.6749	2.0634	0.64212	0.3405	0.1404	0.1954
C1/30	22.27	18.468	1.82215	101.925	0.86805	2.6784	0.65216	0.3376	0.07	0.1936
C1/60	21.25	18.808	1.81924	98.55	0.92745	1.008	0.59819	0.339	0.1268	0.1988
C2/30	24.2	19.208	1.82312	98.55	0.6183	0.4752	0.63067	0.3409	0.1404	0.1804
C2/60	21.31	18.624	1.82263	104.445	0.89325	0.6768	0.61818	0.3379	0.15	0.1984
C3/30	18.87	18.944	1.82457	105.435	0.9729	1.6848	0.60119	0.3407	0.0624	0.2052
C3/60	22.67	18.792	1.81972	101.07	0.74295	1.1808	0.61768	0.34	0.0448	0.1904
E1/30	24.48	18.944	1.81875	105.615	0.80415	0.3456	0.61768	0.3395	0.0364	0.1784
E1/60	21.16	18.4	1.82166	101.61	0.5571	3.6288	0.61568	0.3379	0.0404	0.1928
E2/30	23.97	18.92	1.82457	109.395	0.801	0.8208	0.67315	0.3391	0.242	0.2064
E2/60	21.92	18.42	1.82409	101.79	0.9666	6.0768	0.66915	0.3406	0.212	0.1984
E3/30	22.91	18.98	1.83302	103.54	0.9452	3.5441	0.6689	0.3601	0.233	0.1851
E3/60	22.84	18.77	1.83321	103.83	0.8771	5.4551	0.6681	0.3622	0.2413	0.1924
P1/30	21.24	18.86	1.82457	103.005	0.4482	3.1968	0.62717	0.3399	0.0732	0.2064
P1/60	20.53	18.52	1.82069	91.44	0.6057	5.7024	0.60269	0.3398	0.08456	0.2048
P2/30	21.55	18.42	1.83044	102.52	0.4022	3.1884	0.63046	0.33542	0.08522	0.2034
P2/60	21.37	18.38	1.82043	102.67	0.4322	4.2431	0.54607	0.32254	0.04257	0.1952
P3/30	20.63	18.57	1.8233	103.73	0.6532	4.3256	0.60462	0.32242	0.05623	0.1904
P3/60	20.85	18.62	1.8355	103.55	0.7253	5.872	0.67524	0.31124	0.04223	0.1827

Table (5): Content of main salts in soil samples

SITES	Ca^{++}	Mg^{++}	K^{+}	Na^{+}	Cl^{-}	CO_3^{--}	HCO_3^{-}	SO_4^{--}
Sampling	Cation meq/l				Anion meq/l			
A1/30	0.4	0.2	0.12	0.68	0.7	0	0.3	0.4
A1/60	5.36	0.9	0.12	5.12	7.06	0	0.3	4.14
A2/30	0.55	0.31	0.14	0.8	0.9	0	0.6	0.3
A2/60	0.35	0.21	0.1	0.94	0.7	0	0.3	0.6
A3/30	0.43	0.32	0.13	0.79	0.8	0	0.4	0.56
A3/60	3.55	0.31	0.11	0.92	0.82	0	0.36	0.45
B1/30	0.82	0.32	0.26	1.5	1.6	0	0.7	0.6
B1/60	2.3	1.43	0.23	2.15	3.1	0	0.6	2.4
B2/30	2	0.53	0.18	1.49	2.1	0	0.6	1.5
B2/60	0.82	0.32	0.26	1.3	1.4	0	0.7	0.6
B3/30	1.05	0.55	0.2	1.5	2.73	0	0.4	0.17
B3/60	0.48	0.2	0.1	0.66	0.71	0	0.31	0.42
D1/30	0.46	0.3	0.14	1.2	1.4	0	0.4	0.32
D1/60	0.56	0.3	0.16	1.11	1.17	0	0.64	0.36
D2/30	0.34	0.3	0.2	1.16	1.18	0	0.51	0.31
D2/60	0.35	0.29	0.1	1.06	0.9	0	0.3	0.6
D3/30	0.42	0.33	0.23	1.04	0.85	0	0.31	0.43
D3/60	0.52	0.31	0.22	1.02	0.88	0	0.53	0.47
C1/30	1.2	0.9	0.11	1.6	3.2	0	0.4	0.2
C1/60	0.72	0.32	0.26	1.3	1.4	0	0.6	0.6
C2/30	2.15	1.43	0.22	2.5	3.2	0	0.6	2.5
C2/60	1.9	0.97	0.3	3.43	3.04	0	1.44	2.12
C3/30	3.5	0.8	0.12	8.2	11	0	1.3	0.32
C3/60	4.8	1.74	0.76	10.5	16.2	0	0.8	0.8
E1/30	0.8	0.25	0.11	1.64	1.87	0	0.43	0.56
E1/60	0.4	0.33	0.17	1.4	1.1	0	0.2	1
E2/30	0.73	0.31	0.14	1.32	1.41	0	0.46	0.63
E2/60	0.54	0.26	0.11	1.29	1.35	0	0.64	0.21
E3/30	1.97	0.37	0.13	1.66	1.33	0	0.48	0.57
E3/60	1.87	0.32	0.21	1.84	1.65	0	0.59	0.62
P1/30	2.82	1.24	0.25	4.99	5.4	0	2.83	1.07
P1/60	3.12	1.72	0.23	11.83	12.73	0	1	3.17
P2/30	3.24	1.28	0.21	4.85	6.11	0	2.03	1.09
P2/60	3.37	1.81	0.24	9.56	7.52	0	2.37	2.07
P3/30	3.6	1.77	0.22	10.42	9.45	0	2.4	1.03
P3/60	3.9	1.89	0.32	11.61	11.31	0	2.75	1.98

Table 6. Ismailia wastewater treatment facility laboratory daily/monthly and summary analysis report.

	Influent			Effluent		
	Minimum	Maximum	Average	Minimum	Maximum	Average
January 2020	79,369	252,369	70,405	47,000	220,000	98,000
February 2020	20,369	63,269	53,692	170,000	1,600,000	402,500
March 2020	54,369	92,369	44,869	22,000	920,000	195,091
April 2020	32,369	62,369	27,460	170,000	430,000	257,500
May 2020	54,369	72,369	29,869	22,000	240,000	77,667
June2020	58,369	202,369	110,036	26,000	170,000	84,750
July2020	12,369	52,369	47,119	110,000	320,000	196,250
August 2020	12,369	32,369	28,619	110,000	280,000	187,778
September2020	52,369	72,369	40,147	220,000	2,200,000	762,222
October 2020	42,369	52,369	44,591	110,000	490,000	335,000
Year 2020 Average	240,755	95,459	49,681	100,700	687,000	259,676

Table 7. Analytical parameters included in the evaluated standards for wastewater reuse

Analytical parameters	Spain	Portugal	Italy	Greece	France	Cyprus
	Microbiological parameters					
Escherichia coli	+			+	+	+
Faecal coliforms		+				
Total coliforms				+		
Salmonella sp.	+		+			

Table (8). Assessment of microbial diversity analysis in soil samples and treated wastewater sample

Samples	(TBC) (cfu x 10^9) gm/soil	(TFC) (cfu x 10^6) gm/soil	(TCF) (cfu x 10^5) gm/soil	Actino. (cfu x 10^6) gm/soil	Spore forming (cfu x 10^5) gm/soil	N2 fixers (cfu x 10^4) gm/soil
A1	1.51	1.21	2.74	3.22	2.41	4.60
A2	2.22	1.71	3.68	3.81	3.11	6.03
A3	1.14	1.90	2.34	2.40	2.03	3.82
A4	1.56	0.69	2.88	3.96	2.45	4.34
A5	1.73	1.57	3.12	3.60	2.62	5.51
B1	2.52	1.75	3.96	3.40	3.41	6.63
B2	1.91	1.29	3.28	3.71	2.8	5.49
B3	1.42	1.67	2.64	3.81	2.31	4.42
B4	2.11	1.23	3.68	1.96	3.24	5.80
B5	1.23	1.16	2.42	2.92	2.12	4.01
C1	0.82	1.08	1.92	3.10	1.71	3.21
C2	2.15	1.21	3.56	2.36	3.04	5.88
C3	1.35	1.12	2.56	2.72	2.24	4.28
C4	1.55	0.72	2.79	3.27	2.44	4.63
C5	1.50	0.82	2.76	2.31	2.39	4.58
D1	1.60	1.52	2.86	2.51	2.49	4.78
D2	1.69	1.22	2.96	3.91	2.58	4.97
D3	2.52	1.37	3.96	2.85	3.41	6.63
D4	1.50	1.40	2.76	3.33	2.39	4.58
D5	2.10	1.63	3. 56	2.78	2.99	5.78
E1	1.55	1.74	2.79	2.83	2.44	4.63
E2	1.74	1.39	3. 24	3.52	2.63	5.02
E3	1.65	1.51	2.96	3.20	2.54	4.83
E4	1.87	1.46	3.18	3.15	2.76	5.35
E5	1.19	1.13	2. 64	2.33	2.08	3.97
P1	1.20	0.98	2. 76	3.42	2.09	3.98
P2	1.14	0.85	2.34	1.93	2.03	3.82
P3	1.12	0.87	2.28	2.08	2.01	3.89
AVERAGE	1.62	1.29	2.93	3.01	2.52	4.83
(TSW)	2.95	1.54	4. 76	2.22	3.84	7.43
(TBC): Total bacteria count, (TFC): Total fungi count, (TCF): Total coli form Actino.: Actinomycetes, (CFU): Colony forming units, (TSW): Treated sewage wastewater						

Table (9). Morphological and Biochemical characterization of some serious suspected Bacteria in soil samples

Suspected Bacteria	Morphological characterization							Biochemical tests		
	cell shape	color	Texture	form	gram staining	spores	motility	catalase	oxidase	indole
Escherichia coli	straight rod	Greenish	Rough surface	circular	-	-	+	+	-	+
Pseudomonas spp.	rods	Light yellow	Slightly raisd	circular	-	-	-	+	+	-
Clostridium spp.	convex	whitish	slightly curved	irregular	+	+	+	+	+	-
Salmonella spp.	rods	whitish	mucous	circular	-	-	+	+	-	-
Rhizobium spp.	round	creamy	mucous	irregular	-	-	-	+	+	-

Table (10). Principal component analysis for each location

Location of samples	PCs	Eigenvalues	Proportion	Accumulated proportion
A	PC1	6.792	29.531	29.531
	PC2	5.539	24.083	53.614
	PC3	4.577	19.902	73.516
	PC4	3.678	15.990	89.506
	PC5	2.414	10.494	100.000
B	PC1	7.264	31.582	31.582
	PC2	5.988	26.036	57.618
	PC3	4.502	19.573	77.191
	PC4	3.155	13.719	90.910
	PC5	2.091	9.090	100.000
C	PC1	6.252	27.183	27.183
	PC2	5.860	25.479	52.662
	PC3	5.052	21.967	74.629
	PC4	3.820	16.608	91.238
	PC5	2.015	8.762	100.000
D	PC1	8.482	36.879	36.879
	PC2	7.472	32.485	69.364
	PC3	3.022	13.141	82.505
	PC4	2.542	11.052	93.557
	PC5	1.482	6.443	100.000
E	PC1	8.161	35.481	35.481
	PC2	5.757	25.029	60.509
	PC3	5.114	22.234	82.743
	PC4	2.533	11.012	93.755
	PC5	1.436	6.245	100.000
P	PC1	8.057	35.032	35.032
	PC2	5.749	24.995	60.027
	PC3	4.407	19.160	79.186
	PC4	3.590	15.608	94.794
	PC5	1.197	5.206	100.000

Table (11). Soil quality criteria for each sample site

Samples sites	SQI value	Soil quality criteria
A1/30	0.53	Moderate
A1/60	0.54	Moderate
A2/30	0.58	Moderate
A2/60	0.53	Moderate
A3/30	0.59	Good
A3/60	0.53	Moderate
B1/30	0.59	Moderate
B1/60	0.58	Moderate
B2/30	0.56	Moderate
B2/60	0.58	Moderate
B3/30	0.6	Good
B3/60	0.6	Good
C1/30	0.59	moderate
C1/60	0.74	Good
C2/30	0.64	Good
C2/60	0.63	Good
C3/30	0.69	Good
C3/60	0.63	Good
D1/30	0.52	Moderate
D1/60	0.56	Moderate
D2/30	0.59	Moderate
D2/60	0.55	Moderate
D3/30	0.55	Moderate
D3/60	0.56	Moderate
E1/30	0.64	GOOD
E1/60	0.61	GOOD
E2/30	0.57	MODERATE
E2/60	0.58	MODERATE
E3/30	0.62	GOOD
E3/60	0.61	GOOD
P1/30	0.55	MODERATE
P1/60	0.56	MODERATE
P2/30	0.56	MODERATE
P2/60	0.56	MODERATE
P3/30	0.55	MODERATE
P3/60	0.58	MODERATE